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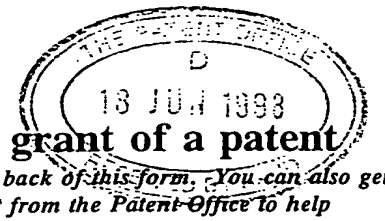

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3. Full name, address and postcode of the or of each applicant *(underline all surnames)* — KAROLINSKA INNOVATIONS AB
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Patents ADP number *(if you know it)* — 7459514001

If the applicant is a corporate body, give the country/state of its incorporation — SE

4. Title of the invention — METHODS AND MEANS FOR MODULATING APOPTOSIS

5. Name of your agent *(if you have one)* — MEWBURN ELLIS

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METHODS AND MEANS FOR MODULATING APOPTOSIS

The present invention relates in various aspects to methods and means for modulating apoptosis and/or cellular proliferation, in particular via stimulation or inhibition of Fas (also known as APO-1 and CD95). It is based in part on the surprising discovery of anti-Fas autoantibodies in human sera, which antibodies moreover are biologically functional. Peptide fragments of Fas and variants and mimetics thereof may be used in modulating apoptosis for therapeutic purposes.

Affinity-purified anti-Fas antibodies isolated from the serum of healthy blood donors have been found by the present inventors to be able to inhibit proliferation and to induce apoptosis of Jurkat leukemia T cells. This effect is inhibited by soluble Fas-Fc chimeric protein. Costimulation of peripheral blood mononuclear cells by human anti-Fas autoantibodies and anti-CD3 monoclonal antibodies induces or inhibits cell proliferation depending on the activation state of the cells. Anti-Fas autoantibodies may thus represent an additional mode of regulation of Fas-mediated signals *in vivo* which may be harnessed in accordance with the present invention.

Fas (also called CD95/APO-1) is a type I cellular receptor protein, belonging to the nerve growth factor/tumor necrosis factor (NGF/TNF) receptor family (Itoh, et al., 1991; Smith et al., 1994). The receptor has been shown to transduce an

multiple sclerosis and liver cirrhosis, and HIV infection. Tachiban *et al* (*Cancer Research* (1995) 55: 5528-5530) have reported correlation between progression of astrocytomas and increased expression of Fas in the tumour cells. De Maria and Testi (*Immunology Today* (1998) 19: 121-125) review evidence of cells expressing Fas and its natural ligand in the proximity of lesions in multiple sclerosis, type I diabetes, liver diseases and HIV infection.

The surprising discovery of anti-Fas autoantibodies in human serum allows for modulation of binding of those antibodies to Fas to modulate Fas-mediated effects, particularly apoptosis. Peptide fragments of Fas, and mimetics thereof, may be used to block antibody binding to Fas, inhibiting or increasing binding of Fas ligand to Fas. Prior to the work of the present inventors, it would not have been reasonable to expect administration of peptide fragments of Fas (a self-antigen) to have any utility.

As noted below in the experimental section, aspects of the present invention are exemplified by peptide fragments of Fas known as Fp5, with sequence GQFCHKPCPPGERKARDCTV corresponding to Gly_{40}-Val_{59} of Fas, Fp11, with sequence EINCTRTQNTKCRCKPNFFC corresponding to Glu_{100}-Cys_{119} of Fas, and Fp17 with sequence WLCLLLLPIPLIVWVKRKEV corresponding to Trp_{160}-Val_{179} of Fas. Fp5 is demonstrated herein to be able to induce apoptosis. Fp11 and Fp17 are demonstrated herein to be able to block apoptosis. Auto-antibodies against Fp11

A peptide for use in the present invention may be a fragment of Fas or may be a variant or derivative thereof, by way of addition, deletion, insertion or substitution of one or more amino acids. Such a variant or derivative thereof will generally retain ability to modulate, either induce or inhibit, apoptosis and/or cellular proliferation (e.g. as measured using Jurkat cells or T-cells).

Preferably, the amino acid sequence of a variant or derivative peptide shares sequence similarity or identity with the relevant Fas fragment sequence, preferably at least about 30%, or 40%, or 50%, or 60%, or 70%, or 75%, or 80%, or 85% similarity or identity, or at least about 90% or 95% similarity or identity. As is well-understood, similarity allows for "conservative variation", i.e. substitution of one hydrophobic residue such as isoleucine, valine, leucine or methionine for another, or the substitution of one polar residue for another, such as arginine for lysine, glutamic for aspartic acid, or glutamine for asparagine. Similarity may be as defined and determined by the TBLASTN program, of Altschul *et al.* (1990) *J. Mol. Biol.* 215: 403-10, which is in standard use in the art. Similarity or identity may be over the full-length of the relevant polypeptide or may more preferably be over a contiguous sequence of about 15, 20, 25, 30, 35 or 40 amino acids, compared with the relevant wild-type amino acid sequence.

A peptide according to the present invention may be provided

A further aspect of the present invention provides the Fp17 peptide, also variants and derivatives thereof that retain the ability to modulate apoptosis and/or cell (e.g. Jurkat or T-cell) proliferation.

Peptides and polypeptides (e.g. fusion molecules including a peptide as discussed) in accordance with the present invention may be made using any of a variety of techniques at the disposal of the ordinary person skilled in the art.

Peptides may be synthesized using standard peptide chemistry such as by the common method employing Fmoc (Fluorenilmetil-ossicarbonil)t-Bu (*tert*-butil), as described in Atherton and Sheppard (1989), *Solid Phase Peptide Synthesis, a Practical Approach*, IRL Press, Oxford.

A convenient way of producing a peptide or polypeptide according to the present invention is to express nucleic acid encoding it, by use of the nucleic acid in an expression system. Accordingly, the present invention also encompasses a method of making a peptide or polypeptide (as disclosed), the method including expression from nucleic acid encoding the peptide or polypeptide (generally nucleic acid according to the invention). This may conveniently be achieved by growing a host cell in culture, containing such a vector, under appropriate conditions which cause or allow expression of the polypeptide. Peptides and polypeptides may also be expressed in *in vitro* systems, such as reticulocyte lysate.

plasmid vectors, are known in the art, see e.g. US Patent No. 5,252,479 and WO 93/07282. In particular, a number of viruses have been used as gene transfer vectors, including papovaviruses, such as SV40, vaccinia virus, herpesviruses, including HSV and EBV, and retroviruses. Many gene therapy protocols in the prior art have used disabled murine retroviruses. A variety of adenovirus and adeno-associated viral vectors have been developed. Alternatives to viral vectors include transfer mediated by liposomes and direct DNA uptake and receptor-mediated DNA transfer.

Host cells containing nucleic acid encoding a peptide or polypeptide (or mixture thereof) according to the present invention may themselves be used in therapeutic or prophylactic treatment of individuals. Such host cells are chosen in order to target the delivery of the nucleic acid encoding the peptide to the relevant site of the body in which target lesions develop. For example, in multiple sclerosis apoptosis of Fas expressing oligodendrocytes may be mediated by an increase in anti-Fp17 auto-antibodies. Provision of Fp17 in the brain may be used to block these antibodies, and this may be achieved using host cells that home to the brain, e.g. macrophages.

Nucleic acid is generally provided as DNA or RNA, though may include one or more nucleotide analogues, and may be wholly or partially synthetic. Nucleic acid molecules and vectors according to the present invention may be provided in

lines available in the art for expression of a heterologous polypeptide include Chinese hamster ovary cells, HeLa cells, baby hamster kidney cells, COS cells and many others. A common, preferred bacterial host is *E. coli*.

A further aspect of the present invention provides a host cell containing nucleic acid as disclosed herein. The nucleic acid of the invention may be integrated into the genome (e.g. chromosome) of the host cell. Integration may be promoted by inclusion of sequences which promote recombination with the genome, in accordance with standard techniques. The nucleic acid may be on an extra-chromosomal vector within the cell.

A still further aspect provides a method which includes introducing the nucleic acid into a host cell. The introduction, which may (particularly for *in vitro* introduction) be generally referred to without limitation as "transformation", may employ any available technique. For eukaryotic cells, suitable techniques may include calcium phosphate transfection, DEAE-Dextran, electroporation, liposome-mediated transfection and transduction using retrovirus or other virus, e.g. vaccinia or, for insect cells, baculovirus. For bacterial cells, suitable techniques may include calcium chloride transformation, electroporation and transfection using bacteriophage. As an alternative, direct injection of the nucleic acid could be employed. Marker genes such as antibiotic resistance or sensitivity

variants and derivatives thereof in accordance with the present invention, including fragments of Fp17 including the C-terminal 10 amino acids, and variants and derivatives thereof.

According to a further aspect of the present invention there is provided a method of obtaining one or more antibody molecules containing a binding site able to bind Fas, the method including bringing into contact a population of antibody molecules and a peptide according to the present invention, and selecting one or more antibody molecules of the population able to bind said peptide.

The method may involve bringing the population of antibodies into contact with a plurality of peptides according to the invention.

The peptide or peptides may be administered to a non-human mammal to bring them into contact with a population of antibody molecules produced by the mammal's immune system, then one or more antibody molecules able to bind the peptide or peptides may be taken from the mammal, or cells producing such antibody molecules may be taken from the mammal. The mammal may be sacrificed.

If cells are taken from the mammal, antibody molecules may be taken from said cells or descendants thereof. Such descendants in particular may include hybridoma cells.

administering a peptide or polypeptide or mixture of peptides or polypeptides to a mammal in order to raise an antibody response. In a therapeutic or prophylactic context the mammal may be human or non-human. For the production of antibodies or antibody-producing cells to be isolated and used for any of a variety of purposes, a step of sacrificing a non-human mammal may be included. Such a non-human mammal may be for example mouse, rat, rabbit, dog, cat, pig, horse, donkey, goat, sheep, camel, Old World monkey, chimpanzee or other primate. Antibodies may be obtained from immunized animals using any of a variety of techniques known in the art, and screened, preferably using binding of antibody to peptide or polypeptide of interest. For instance, Western blotting techniques or immunoprecipitation may be used (Armitage et al, Nature, 357:80-82, 1992).

The production of polyclonal and monoclonal antibodies is well established in the art. Monoclonal antibodies can be subjected to the techniques of recombinant DNA technology to produce other antibodies or chimeric molecules which retain the specificity of the original antibody. Such techniques may involve introducing DNA encoding the immunoglobulin variable region, or the complementarity determining regions (CDRs), of an antibody to the constant regions, or constant regions plus framework regions, of a different immunoglobulin. See, for instance, EP-A-184187, GB-A-2188638 or EP-A-239400. Humanized antibodies in which CDRs from a non-human source are grafted onto human framework regions,

antigen or epitope. Example antibody fragments, capable of binding an antigen or other binding partner are the Fab fragment consisting of the VL, VH, Cl and CH1 domains; the Fd fragment consisting of the VH and CH1 domains; the Fv fragment consisting of the VL and VH domains of a single arm of an antibody; the dAb fragment which consists of a VH domain; isolated CDR regions and $F(ab')_2$ fragments, a bivalent fragment including two Fab fragments linked by a disulphide bridge at the hinge region. Single chain Fv fragments are also included.

Hybridomas capable of producing antibody with desired binding characteristics are within the scope of the present invention, as are host cells, eukaryotic or prokaryotic, containing nucleic acid encoding antibodies (including antibody fragments) and capable of their expression. The invention also provides methods of production of the antibodies including growing a cell capable of producing the antibody under conditions in which the antibody is produced, and preferably secreted.

The reactivities of antibodies on a sample (e.g. in a diagnostic test) may be determined by any appropriate means. Tagging with individual reporter molecules is one possibility. The reporter molecules may directly or indirectly generate detectable, and preferably measurable, signals. The linkage of reporter molecules may be directly or indirectly, covalently, e.g. via a peptide bond or non-

general knowledge.

Antibodies according to the present invention may be used in screening for the presence of a peptide or polypeptide, for example in a test sample containing cells or cell lysate as discussed, and may be used in purifying and/or isolating a peptide or polypeptide according to the present invention, for instance following production of the polypeptide by expression from encoding nucleic acid therefor.

Antibodies are also useful in prophylaxis, by way of passive immunisation, and in therapy. Where antibodies are to be administered, it may be preferable to include a mixture of antibodies, such as antibodies collectively cross-reactive with a plurality of peptides according to the present invention.

Antibodies which bind a peptide in accordance with the present invention may themselves be used as immunogens in the production of anti-idiotypic antibodies. These may be used to mimic a peptide epitope in raising an immune response in an individual, e.g. for therapeutic and/or prophylactic purposes.

An antibody may be provided in a kit, which may include instructions for use of the antibody, e.g. in determining the presence of a particular substance in a test sample. One or more other reagents may be included, such as labelling

binding of antibody to the peptide and/or binding of antibody to Fas and/or ability of binding of antibody to Fas to induce or inhibit Fas-mediated apoptosis.

For instance, a method according to one aspect of the invention includes providing a peptide or antibody of the invention and bringing it into contact with a substance, which contact may result in binding between the peptide or antibody and the substance. Binding may be determined by any of a number of techniques available in the art, both qualitative and quantitative.

In various aspects the present invention is concerned with provision of assays for substances which inhibit interaction between a peptide of the invention and an antibody directed against it.

Further assays are for substances which interact with or bind a peptide of the invention.

One aspect of the present invention provides an assay which includes:

(a) bringing into contact a peptide according to the invention and a putative binding molecule or other test substance; and

(b) determining interaction or binding between the polypeptide or peptide and the test substance.

Such an assay may include determination of interaction between Fas and antibody, with a peptide according to the invention also being presence, the assay determine the effect of the test substance on ability of the peptide to modulate interaction between Fas and antibody.

The precise format of an assay of the invention may be varied by those of skill in the art using routine skill and knowledge. For example, interaction between a peptide and another molecule such as an antibody may be studied *in vitro* by labelling one with a detectable label and bringing it into contact with the other which has been immobilised on a solid support, such as a plastic surface. Suitable detectable labels include ^{35}S-methionine which may be incorporated into recombinantly produced peptides and polypeptides. Other labels or markers include alkaline phosphatase, peroxidase, avidin-biotin, which may be coupled directly to an anti-peptide antibody. A further option for those skilled in the art is to use a labelled anti-anti-peptide antibody which may be reacted to peptide-anti-peptide. Activity of alkaline phosphatase, peroxidase or avidin-biotin, or other label, may be measured in a spectrophotometer.

Further assays according to aspects of the present invention involve determination of the ability of a test substance to modulate Fas-mediated apoptosis of cells.

The binding of antibody to Fas, e.g. present on a cell

Combinatorial library technology (Schultz, JS (1996) Biotechnol. Prog. 12:729-743) provides an efficient way of testing a potentially vast number of different substances for ability to modulate Fas activity. Prior to or as well as being screened for modulation of activity, test substances may be screened for ability to interact with a peptide or antibody of the invention, e.g. in a yeast two-hybrid system. This may be used as a coarse screen prior to testing a substance for actual ability to modulate Fas activity.

The amount of test substance or compound which may be added to an assay of the invention will normally be determined by trial and error depending upon the type of compound used. Typically, from about 0.01 to 100 nM concentrations of putative inhibitor compound may be used, for example from 0.1 to 10 nM. Greater concentrations may be used when a peptide is the test substance.

Compounds which may be used may be natural or synthetic chemical compounds used in drug screening programmes. Extracts of plants which contain several characterised or uncharacterised components may also be used. Other candidate inhibitor compounds may be based on modelling the 3-dimensional structure of a polypeptide or peptide fragment and using rational drug design to provide potential inhibitor compounds with particular molecular shape, size and charge characteristics.

Non-peptide "small molecules" are often preferred for many *in vivo* pharmaceutical uses. Accordingly, a mimetic or mimic of the substance (particularly if a peptide) may be designed for pharmaceutical use. The designing of mimetics to a known pharmaceutically active compound is a known approach to the development of pharmaceuticals based on a "lead" compound. This might be desirable where the active compound is difficult or expensive to synthesize or where it is unsuitable for a particular method of administration, e.g. peptides may not be well suited as active agents for oral compositions as they may be quickly degraded by proteases in the alimentary canal. Mimetic design, synthesis and testing may be used to avoid randomly screening large number of molecules for a target property.

There are several steps commonly taken in the design of a mimetic from a compound having a given target property. Firstly, the particular parts of the compound that are critical and/or important in determining the target property are determined. In the case of a peptide, this can be done by systematically varying the amino acid residues in the peptide, e.g. by substituting each residue in turn. These parts or residues constituting the active region of the compound are known as its "pharmacophore".

Once the pharmacophore has been found, its structure is modeled to according its physical properties, e.g. stereochemistry, bonding, size and/or charge, using data from

use of a peptide as disclosed in the identification or design of a non-peptidyl mimetic which retains ability to modulate apoptosis and/or cell proliferation. A further aspect provides a method of testing a non-peptidyl mimetic of a peptide for use in the present invention for ability to modulate apoptosis and/or cellular proliferation.

As noted already, peptides, mimetics, polypeptides, antibodies and nucleic acid in accordance with the present invention may be formulated into compositions, and are useful in pharmaceutical contexts. These compositions may include, in addition to one of the above substances, a pharmaceutically acceptable excipient, carrier, buffer, stabiliser or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material may depend on the route of administration, e.g. oral, intravenous, cutaneous or subcutaneous, nasal, intramuscular, intraperitoneal routes.

Compositions for oral administration may be in tablet, capsule, powder or liquid form. A tablet may include a solid carrier such as gelatin or an adjuvant. Liquid pharmaceutical compositions generally include a liquid carrier such as water, petroleum, animal or vegetable oils, mineral oil or synthetic oil. Physiological saline solution, dextrose or other saccharide solution or glycols such as

Whether it is a polypeptide, antibody, peptide, nucleic acid molecule, mimetic, small molecule or other pharmaceutically useful compound according to the present invention that is to be given to an individual, administration may be in a "prophylactically effective amount" or a "therapeutically effective amount" (as the case may be, although prophylaxis may be considered therapy). Most preferably the effect is sufficient to prevent the individual from suffering one or more clinical symptoms, and/or reduce pain. A therapeutic effect is sufficient to potentiate the immune response of an individual to pre-existing disorder, preferably sufficient to antagonise the disorder, wholly or partially. Most preferably the effect is sufficient to ameliorate one or more clinical symptoms, and/or cure the disorder and/or reduce pain in the individual. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of what is being treated. Prescription of treatment, e.g. decisions on dosage etc, is within the responsibility of general practitioners and other medical doctors, and typically takes account of the disorder to be treated, the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners. Examples of the techniques and protocols mentioned above can be found in Remington's Pharmaceutical Sciences, 16th edition, Osol, A. (ed), 1980.

Further aspects of the invention provide methods of treatment including administration of a peptide, mixture of peptides,

Another aspect provides a method of treating a mammal against such a disorder, the method including administering a peptide or mixture of peptides, mimetic or mimetics, antibody or antibodies or nucleic acid as disclosed, to the mammal.

A peptide, antibody or other therapeutic molecule according to the present invention may be targeted to a lesion, e.g. tumour. Antibodies diffuse rather well through tissues and injection at a site distal to that of the lesion may be employed. Alternatively, direct injection into a lesion, e.g. tumour, may be employed, and this may be utilised for peptides and other molecules. Targeted viral vectors may be used to deliver nucleic acid encoding a peptide, polypeptide or antibody according to the invention to a site for expression of the encoded product.

Further aspects and embodiments of the present invention will be apparent to those skilled in the art based on the present disclosure. Embodiments of and experimental basis for the present invention will now be described in more detail with reference to the following figures:

Figure 1 illustrates results of experiments demonstrating that sera from healthy blood donors contain antibodies against human Fas peptides. Serum specimens from 30 individuals were analyzed. ELISA titers of antibodies against the reactive Fas peptides Fp5, Fp11 and Fp17 are expressed as percentiles and median values by use of the box

Figure 4(B) shows apoptosis of non-stimulated PBMC in response to stimulation with anti-Fas peptide autoantibodies in absence (filled) or presence (open) of anti-CD3 mAb.

Figure 5 illustrates a molecular model of extracellular domain organization of human Fas monomer. The filled volumes represent the side chains of the amino acid stretches involved in the binding of the human anti-Fas peptide auto-antibodies. The side chains of the amino acids which are important in the Fas-FasL interaction (Starling et al., 1997) are visualized as rods. The position of the Fp17, presumed to be part of the transmembrane domain of Fas, is depicted as a separate sequence adjacent to the C-terminal end of the Fas model.

EXPERIMENTAL

Synthesis of Fas peptides.

The derived amino acid sequence (a.a.1-179) of the Fas protein (Itoh et al., 1991) was used for simultaneous multiple solid-phase peptide synthesis (Houghten et al, 1985) of 20 amino acid long peptides (with a 10-residue overlap). The peptides demonstrated 70-97% homogeneity, as revealed by HPLC. These peptides were used in ELISA of 30 human sera obtained from healthy blood donors and the specificity of the assay was confirmed by peptide competition ELISA as described (Leonov et al., 1994). Peptides Fp5 (Gly_{40}-Val_{59}), Fp11 (Glu_{100}-Cys_{119}), and Fp17 (Trp_{160}-Val_{179}) were selected as positively reacting with human sera and were used in

diluted by 1/50 in PBS containing 5% non-fat dry milk, 0,1% Tween-20 and 0,001% anti-foam agent (Sigma).

Bound antibodies were revealed by sequential use of $F(ab)_2$ fragments of goat anti-human light chain antibodies conjugated with peroxidase and SuperSignal ULTRA chemiluminescent substrate system (both are from Pierce, Rockford, IL).

Detection of apoptosis

Jurkat leukemia T cells ($2x10^6$/well) in RPMI 1640 supplemented with 10% FCS, 10 mM-HEPES, 2mM-glutamin, 50 μM-2-mercaptoethanol, 0,1mM-non-essential amino acids, and 10μg/ml gentamicin (RPMI-HEPES) were cultured in 24-well plates previously coated overnight with anti-Fas peptide antibodies isolated by affinity-column. The antibody-concentrations were anti-Fp5 (10μg/ml), anti-Fp11 (10μg/ml) and anti-Fp17 (40 μg/ml). Cells were collected after 48 h incubation, and the fragmented DNA of apoptotic cells was assessed by Apoptosis Detection System, Fluorescein (Promega, Madison, WI) based on TdT-mediated dUTP Nick-End Labeling (TUNEL) assay and FACS analysis (CellQuest software, Becton Dickinson) according to manufacturer's instructions. For apoptosis inhibition experiments, Jurkat cell culture medium was supplemented with 200 ng/ml of recombinant Fas-Fc chimera (R&D Systems Europe Ltd, Abingdon, UK). Inhibition of CH-11-mediated apoptosis was performed as described in Fadeel et al., 1997. Data are presented as the mean value of four

in fluorescence between antibody containing and antibody-free cultures.

Computer-modelling of Fas

The molecular model for the extracellular domain (Itoh et al., 1991) of the Fas monomer (amino acids His_{38}-Lys_{149}) was created using knowledge-based protein modeling methods as implemented in the Swiss-Model server (Peitsch et al., 1995 and 1996). The model was based on the three-dimensional structure of the TNFR1 (entry No. 1TNR in Brookhaven Protein Data Bank).

RESULTS

Anti-Fas antibodies of IgG class are present in the serum of blood donors.

Peptides corresponding to the extracellular and transmembrane parts of human Fas (Itoh, et al., 1991) were synthesized. Three of these peptides, Fp5 (Gly_{40}-Val_{59} with sequence GQFCHKPCPPGERKARDCTV), Fp11 (Glu_{100}-Cys_{119} with sequence EINCTRTQNTKCRCKPNFFC) and Fp17 (Trp_{160}-Val_{179} with sequence WLCLLLLPIPLIVWVKRKEV), were reactive with antibodies present in the blood of the 30 healthy donors (Figure 1). The serum titers against the three different peptides were variable with the lowest mean titers detected against Fp17.

These antibodies were purified by affinity chromatography based on immobilized Fas peptides. Polyacrylamide gel electrophoresis (PAGE) analysis of the eluates demonstrated

be mediated through cross-linking of Fas molecules on the cell surface, a mechanism suggested to operate for anti-Fas monoclonal antibodies of both IgM and IgG subclasses, including IgG1 (Dhein et al., 1992; Fadeel et al., 1997).

Two sets of blocking experiments were performed to establish that apoptosis induced by anti-Fas autoantibodies is mediated through Fas.

The chimeric protein Fas-Fc, consisting of the extracellular part of Fas (aa 1-173) and Fc-part of human IgG was previously shown to block apoptosis caused by Fas-FasL interaction (Itoh et al., 1991). Addition of this protein to Jurkat cells completely reduced apoptosis induced by human antibodies to Fp5 and Fp11 (Figure 2(A)). For Jurkat cells incubated with anti-Fp17, the reduction of apoptosis was 60%, although it can be noted that the portion of Fas included in the Fas-Fc fragment is 1-173 whereas Fp17 span is between Trp_{160} snd Val_{179}.

In the second set of experiments purified anti-Fas autoantibodies in soluble form were used to block apoptosis induced by the anti-Fas IgM monoclonal CH-11. Anti-Fas IgG1 murine monoclonal antibodies were previously demonstrated to inhibit the effect of CH-11 (Fadeel et al., 1997). The autoantibodies directed to Fp-5 and Fp-11 reduced CH-11-mediated apoptosis by more than 50%, thus to levels similar to the spontaneous apoptosis occurring in the non-treated

The auto-antibodies had no effect when immobilized alone (Figure 3(A)). However, costimulation of cells with immobilized anti-CD3 antibodies and anti-Fas autoantibodies significantly reduced proliferation (21-42% reduction) in comparison with anti-CD3 alone (12% reduction). Anti-Fas autoantibodies can therefore enhance CD3-mediated anti-proliferative effect.

Interestingly, it was found that reduction of proliferation was paralleled with increased expression of FasL (Figure 3(B)). The data suggest that costimulation of the CD3/TCR complex and Fas by anti-CD3 and human anti-Fas autoantibodies might decrease the proliferation of activated T cell blasts. A possible mediator of this effect may be FasL, as demonstrated by up-regulation of this molecule upon cross-linking of Fas and CD3.

CD3/TCR and Fas engagement in cellular proliferation and apoptosis was further investigated in experiments with non-stimulated peripheral blood mononuclear cells (PBMC). In this system immobilized human anti-Fas auto-antibodies alone or in combination with anti-CD3 increased proliferation between 20 and 40% (Figure 4(A)). This effect may be due to reduction of spontaneous apoptosis. Indeed, the costimulation with anti-CD3 antibody and human anti-Fas auto-antibodies induced reduction of spontaneous apoptosis by 15 to 55% for the three anti-peptide antibodies (Figure 4(B)). These data correlate with previous observations that CD3 and

anti-Fas autoantibodies on cells by either interference with FasL binding or by direct induction of apoptotic or proliferative signals. In this context it is important to note that the apoptotic activity of the prototypic anti-human Fas mAb (clone CH-11) depends on binding to the region represented by Fp11, thus indicating that this segment display pronounced antigenic property (Fadeel et al., 1995). Of course performance of aspects of the present invention does not require knowledge of how the invention works and the scope of the invention is not limited by any particular theory.

The region of Fas represented by Fp17 (Trp_{160}-Val_{179}) is predicted to be a transmembrane domain and could not be included in the model. The recognition of this Fas domain by auto-antibodies mediating biological effect indicates that part of this domain is likely to be exposed on the cell surface. The titers against Fp17 were the lowest among the three peptides. However, the apoptosis-inducing effect of these anti Fp17 antibodies was evident and exceeded that of anti-Fp5 and anti Fp11 when used at the same concentration. Fp17 does not contain residues involved in the contact with FasL. The results provide indication that autoantibody binding to this Fp17 region may efficiently transduce a signal to the intracellular cell death machinery.

DISCUSSION

The experimental work described above demonstrates the

immune responses through AICD (Kabelitz et al., 1993) and in the maintenance of peripheral tolerance (Fisher et al., 1995; Rieux-Laucat et al., 1995).

The activity of anti-Fas autoantibodies has potential implications in diseases which are linked to Fas dysregulation, e.g. liver damage (Galle et al., 1995), insulin-dependent diabetes mellitus (Stassi et al., 1997) and multiple sclerosis (Dowling et al., 1996; D'Souza et al., 1996). Increased levels of Fas and FasL in these diseases may be paralleled by changes in the levels of Fas autoantibodies.

Autoantibodies to the three regions of Fas display different biological activity. Anti Fp17 autoantibodies are efficient in mediating apoptosis of Jurkat cells whereas anti Fp5 antibodies were found to be poor inducers of apoptosis but can efficiently block the apoptotic activity of anti-fas mAbCH-11. Thus the binding of autoantibodies to regions of Fas which may interfere spacially with the ligand (Fp5) or directly transduce apoptotic signals (Fp17) may be exploited in therapeutical settings aimed at inducing or reducing Fas-mediated apoptosis.

Measurement of distribution and titres of antibodies directed to Fas peptides in accordance with the present invention provides further insight into Fas

Animal models representative of (i) multiple sclerosis and (ii) type I diabetes are treated with a peptide or antibody of the invention in order to block anti-Fas auto-antibody triggering of apoptosis in diseased organs.

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All documents mentioned anywhere herein are incorporated by reference.

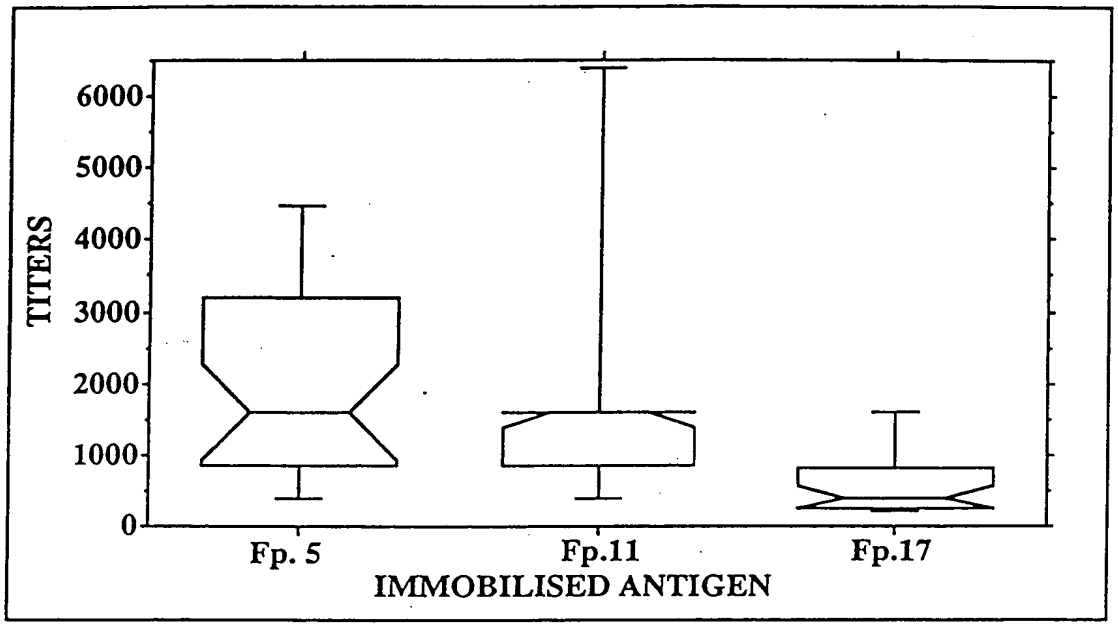

Figure 1

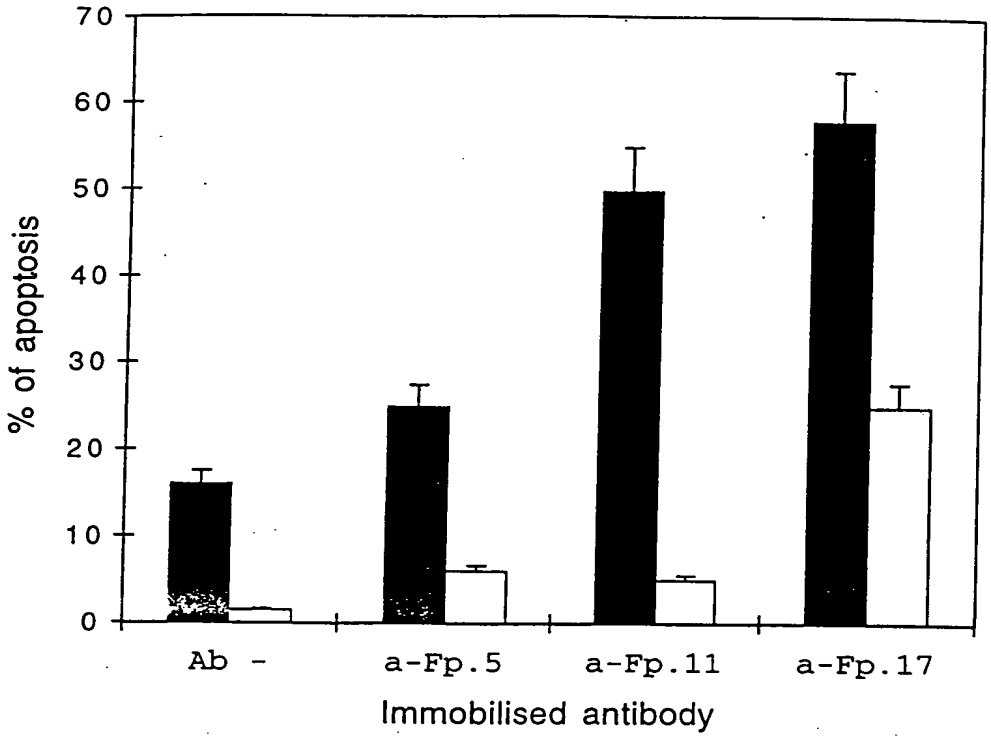

Figure 2a

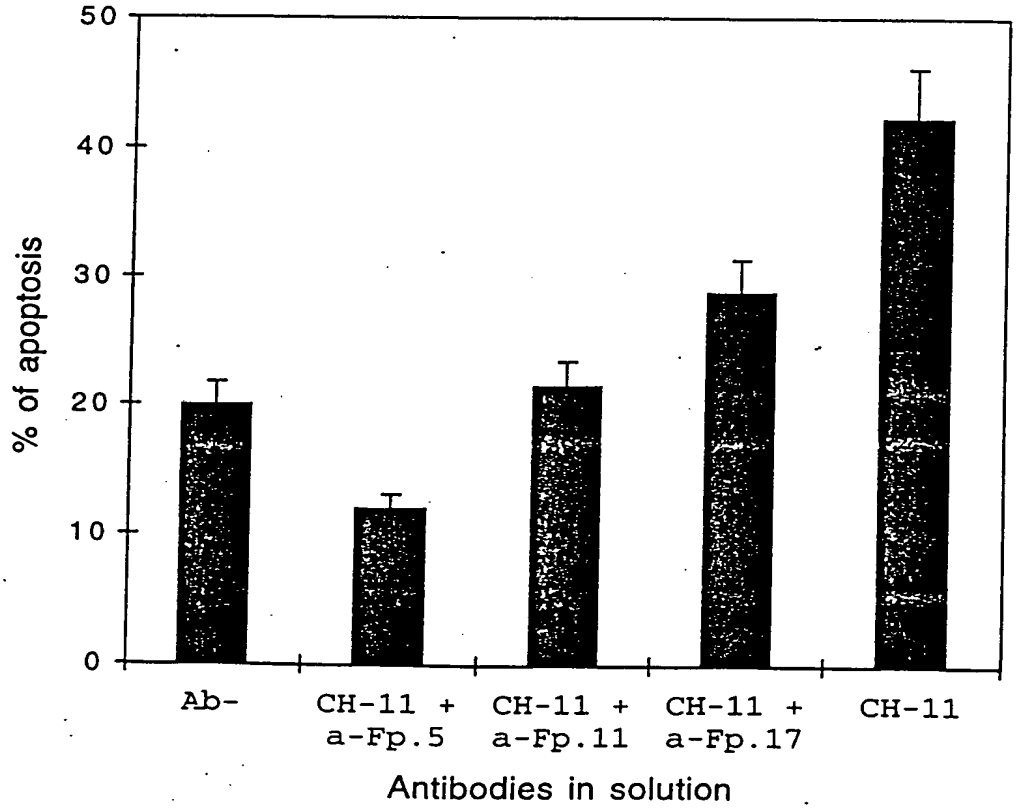

Figure 2b

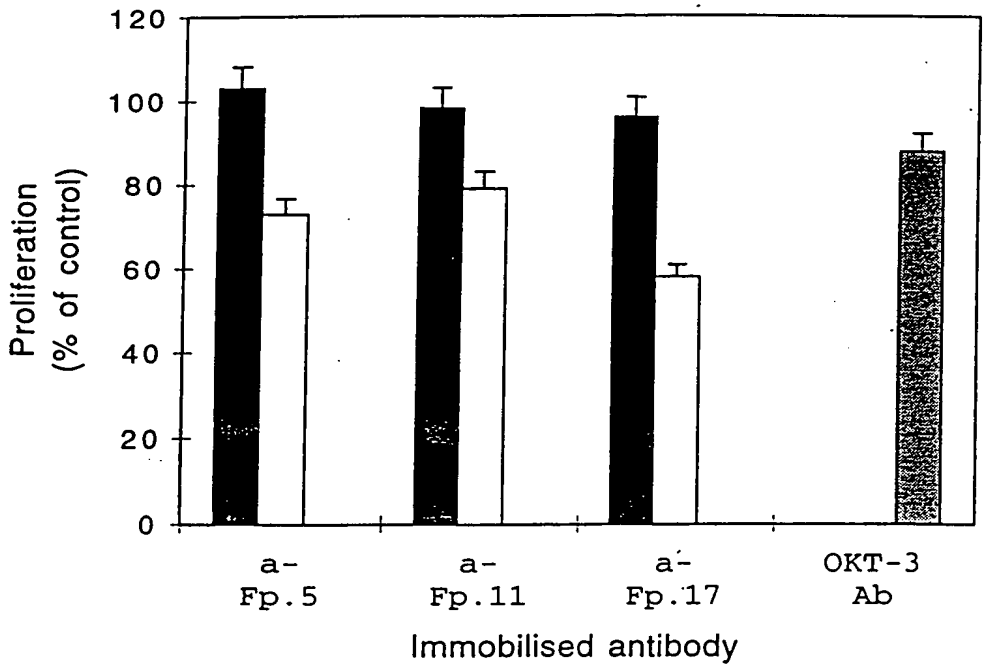

Figure 3a

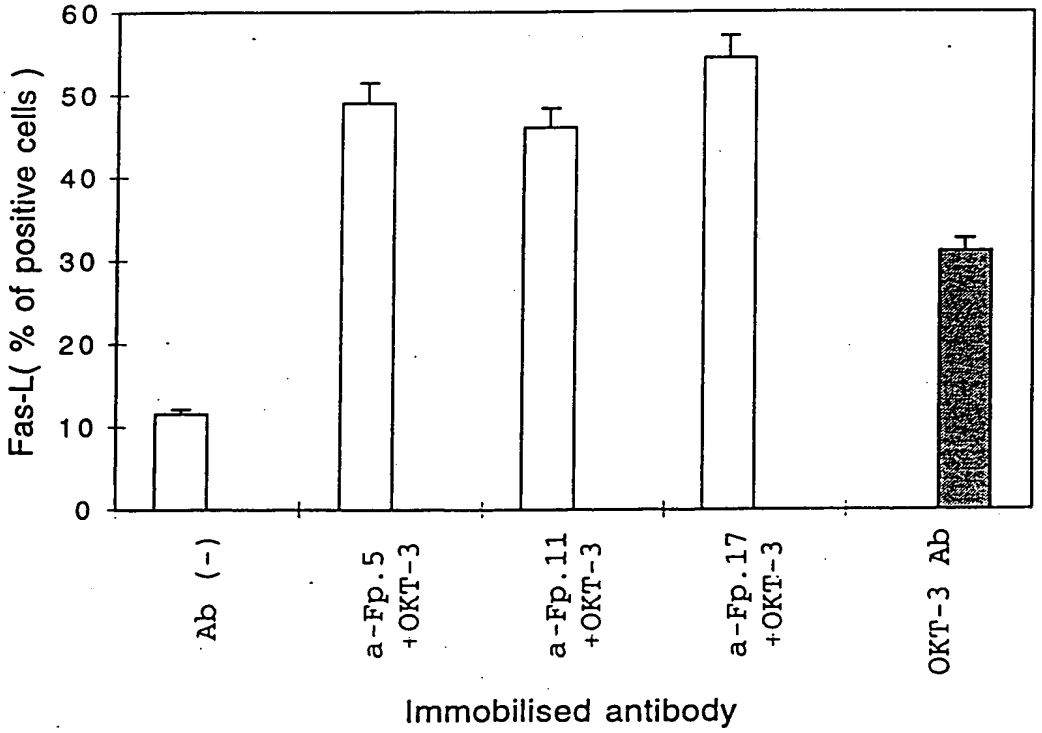

Figure 3b

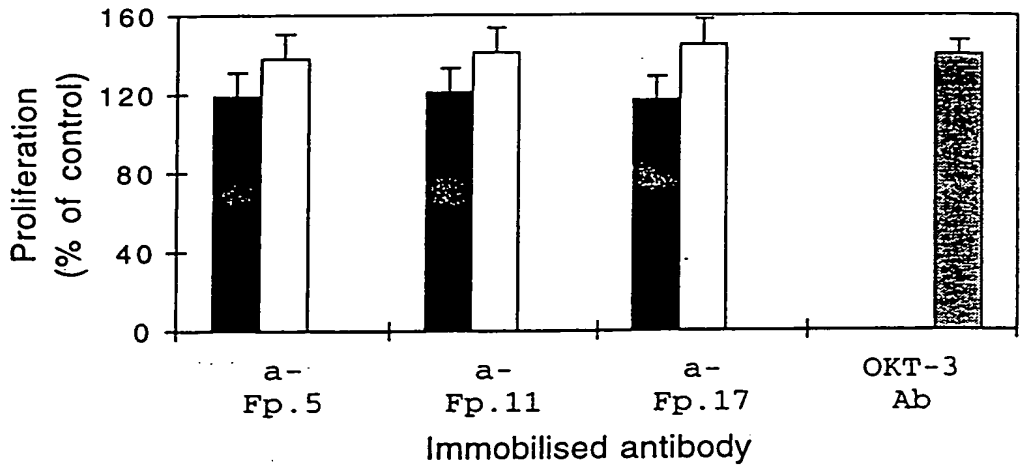

Figure 4a

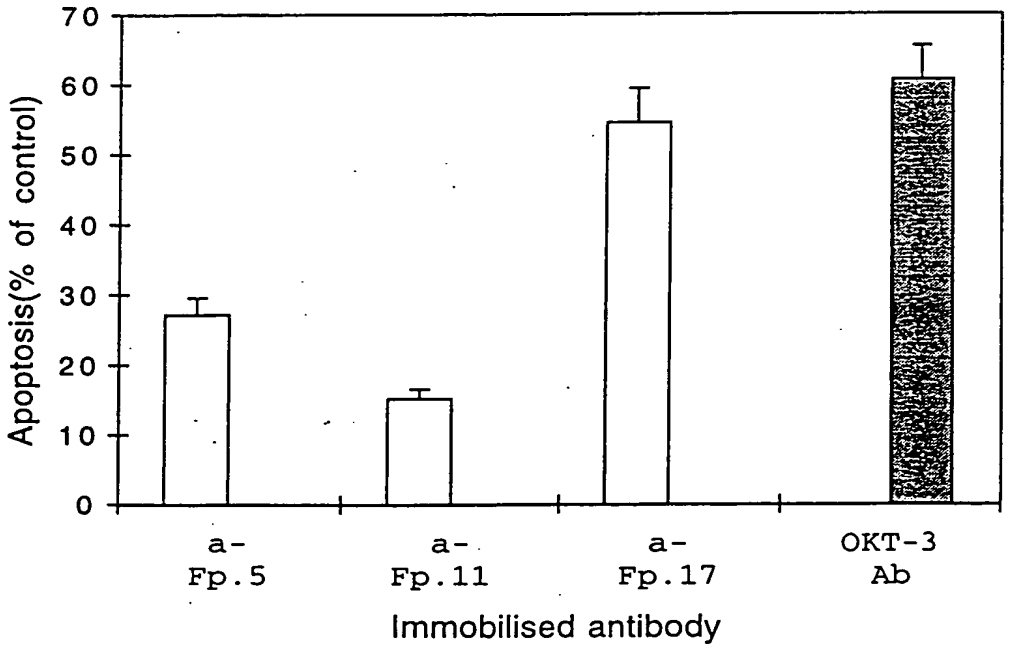

Figure 4b

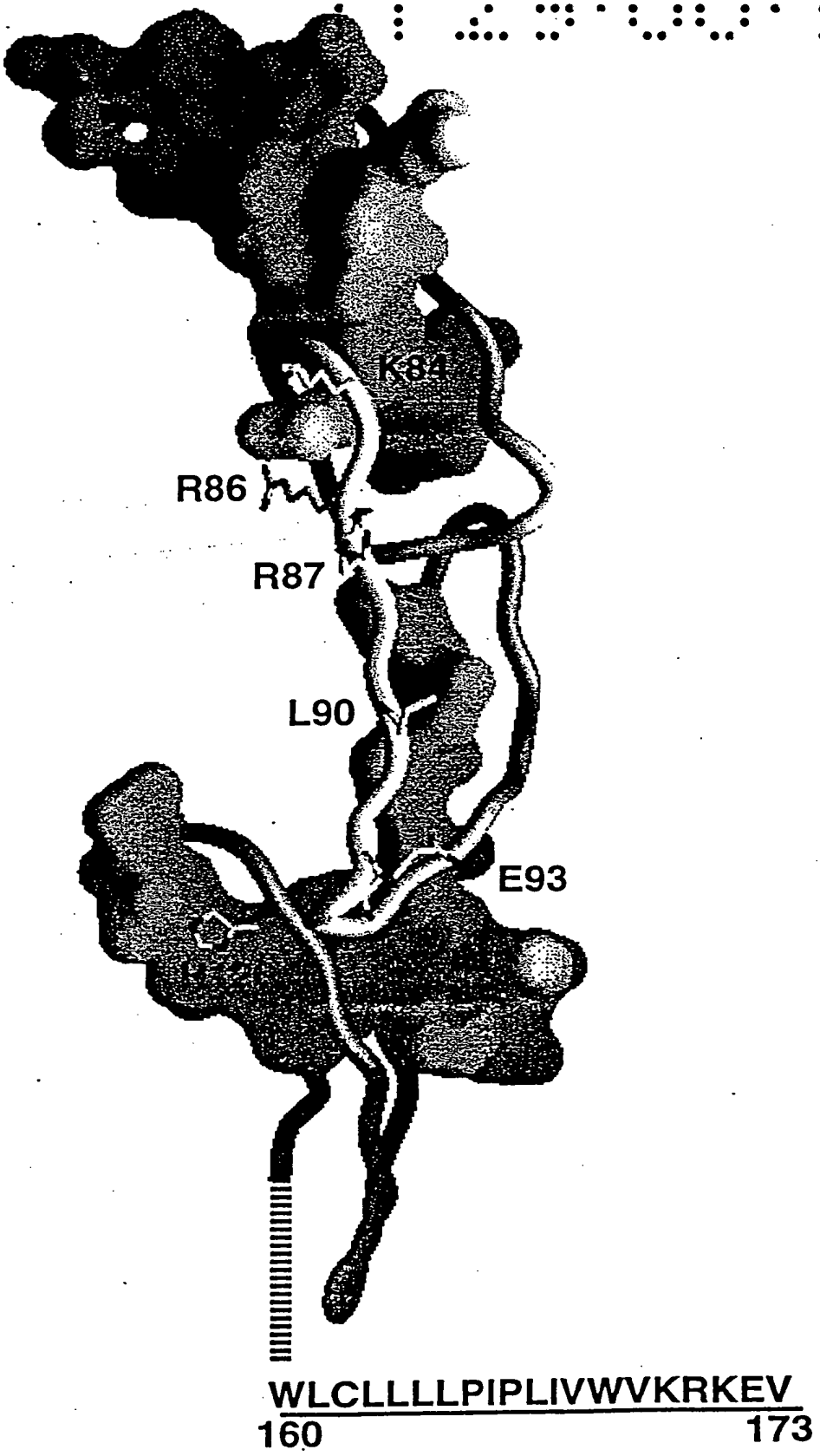

Figure 5